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Raison d'être and structural model for the B-Z transition of poly d(G-C)*poly d(G-C)

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In DNA oligonucleotides crystallized in the A form, the nucleotides adopt standard conformation except for steps 5′-CpG-3′ where reduced base-pair twist and a sliding motion of the base pairs along their long axes causes pronounced interstrand guanine-guanine overlap. As a consequence, torsion angles α, β and γ are consistently *trans*, *trans*, *trans* instead of the common *−gauche*, *trans*, *+gauche*. This conformation significantly increases the intraresidue distance between the guanine base and the 5′-phosphate group. A molecular model of poly d(G-C)·poly d(G-C) built with these structural characteristics in the A form, which we call A^2-DNA, shows that rotation of the guanosine sugar into the *syn* orientation is easily achieved and pushes the base pair across the helix axis. If successive guanosines are changed this way, a smooth transformation occurs to the left-handed Z-DNA. We suggest that A- and A^2-DNA forms of poly d(G-C)·poly d(G-C) are metastable and that the actual transition is B $\rightleftharpoons$ (A$\rightleftharpoons A^2$) $\rightleftharpoons$ Z-DNA.

DNA, A-; DNA, B-; DNA, Z-; B-Z transition; Poly d(G-C)·poly d(G-C)

1. INTRODUCTION

Poly d(G-C)·poly d(G-C) adopts the canonical right-handed double helical B-DNA form in solution under 'normal' low-salt conditions and in the fiber state as Li^+ salt at 81% relative humidity [1–3]. If in an aqueous solution of poly d(G-C)·poly d(G-C) the salt concentration is raised beyond 2.5 M NaCl or 0.7 M $MgCl_2$, or if mM amounts of $Co(NH_3)_6^{3+}$ or 60% alcohol are added, a direct B⟶Z transition is observed without indication of an A form [4,5]. The Z-DNA form is also found in fibers under high-salt conditions [2,3]. NMR data suggest that the B⟶Z transition does not involve opening or breaking of the G-C Watson-Crick base pairs [6].

The A form of poly d(G-C)·poly d(G-C) is usually not detected and it appears that the B⟶Z transition is a direct two-state process [5]. However, the A form was trapped in fibers of the Na^+ salt up to 92% relative humidity [2,3], and it was monitored by CD spectroscopy if trifluoroethanol was added to an aqueous solution of poly d(G-C)·poly d(G-C) [7]. Under these latter conditions, slow transitions A$\rightleftharpoons$Z and B$\rightleftharpoons$Z were observed and a fast B$\rightleftharpoons$A transition which occurred in about 10 s. An A$\rightleftharpoons$Z transition has been described in the ribo series for poly r(G-C)·poly r(G-C) [8].

At present, there are two hypothetical models describing the pathway of the B$\rightleftharpoons$Z transition of poly d(G-C)·poly d(G-C) [9,10]. They both involve vertical base-pair separation followed by 180° flips of the base pairs to induce the typical conformations of Z-DNA: guanosine *syn*, $C_{3'}$-*endo* pucker and cytidine *anti*, $C_{2'}$-*endo*. Based on our own observations with crystallographically determined oligonucleotides containing CpG steps and occurring in the A form [11,12], we arrived at a basically different model which, although not experimentally proven, explains some of the structural and physical peculiarities of poly d(G-C)·poly d(G-C).

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2. THE UNIQUE CpG STEP IN A-DNA

We have made a survey of those DNA fragments analyzed by X-ray crystallography which represent purely Watson-Crick base-paired A-DNA without chemical modifications of the bases and for which structural data are published [11,13–21]. In table 1 are summarized the torsion angles of these oligonucleotides averaged for every dinucleotide step along with torsion angles of double helical DNA in the canonical A-, B- and Z-forms [22]. The torsion angles of all nucleotides in A- and B-DNA are in the normally observed ranges with sequence α *−gauche*, β *trans*, γ *+gauche*, δ *+gauche*, ϵ *trans*, and ζ *−gauche* with the exception of G in the step 5′-CpG-3′ in most A-DNA oligonucleotides in which α and γ are consistently *trans* instead of *−gauche* and *+gauche*, respectively [11,13,15]. According to potential energy calculations, the α *trans* torsion angle corresponds to a local minimum approximately 5 kcal/mol above the global minimum where α and ζ are both *−gauche* [23–25]. Associated with the conformational changes of torsion angles α and γ in the guanosine-5′-phosphate units in A-DNA is a wide intranucleotide separation of the guanine base from $O_{5'}$ so that the intranucleotide distance $O_{5'}$–C_8 increases from the normal 3.4 Å to 4.6 Å (1 Å = 0.1 nm). On the opposite side of the base pair, the (cytidine)C_6–$O_{5'}$ intranucleotide distance remains at 3.2 Å. The Watson-Crick G-C

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Table 1

Average backbone torsion angles in A-DNA helices

	C4'-C3' δ	C3'-O3' ϵ	O3'-P ζ	P-O5' α	O5'-C5' β	C5'-C4' γ	C4'-C3' δ
AA				not observed			
AC	90 ± 3	−150 ± 3	−81 ± 13	−48 ± 28	163 ± 11	40 ± 21	89 ± 1
AG	78 ± 17	−174 ± 13	−54 ± 1	−96 ± 3	−160 ± 13	69 ± 19	92 ± 13
AT	81 ± 8	−158 ± 23	−72 ± 30	−73 ± 16	168 ± 14	57 ± 8	84 ± 8
CA				not described			
CC	80 ± 10	−166 ± 15	−63 ± 11	−88 ± 30	−178 ± 8	69 ± 27	85 ± 13
CG	**79 ± 2**	**−170 ± 7**	**−71 ± 9**	**165 ± 22**	**−169 ± 11**	**167 ± 21**	**81 ± 12**
CT	68 ± 21	173 ± 23	−45 ± 21	−72 ± 8	177 ± 10	46 ± 16	76 ± 15
GA	76 ± 7	−168 ± 13	−61 ± 6	−79 ± 15	−163 ± 6	51 ± 13	81 ± 10
GC	86 ± 15	−154 ± 14	−76 ± 17	−64 ± 23	168 ± 11	56 ± 13	90 ± 27
GG	85 ± 14	−154 ± 15	−70 ± 12	−84 ± 29	−179 ± 11	69 ± 26	82 ± 10
GT	87 ± 0	−141 ± 3	−83 ± 1	−52 ± 1	178 ± 8	35 ± 4	91 ± 5
TA	84 ± 11	−162 ± 17	−73 ± 17	−89 ± 64	179 ± 19	77 ± 50	84 ± 10
TC	84 ± 10	−162 ± 8	−71 ± 6	−71 ± 12	173 ± 15	55 ± 11	75 ± 7
TG				not described			
TT				not observed			
A-DNA	79	−148	−75	−52	175	42	79
B-DNA	143	−141	−161	−30	136	31	143
Z-DNA							
CG	138	−97	82	52	179	−174	95
GC	95	−104	−65	−140	−137	51	138

Angles are given in degrees between −179° and 180°. Data taken from [11,13,15–19,21,22]. The CpG step is emphasized by bold-face print

base pair is pushed along its C_8–C_6 axis and, since the adjacent base pair is moved the other way, the guanine-guanine stacking overlap is increased (fig.1). We assume that these stacking interactions are the driving force for the lateral shifts of the G-C base pairs and induce the *−gauche* and *+gauche* ⟶ *trans* change of torsion angles α and γ of the guanosine-5'-phosphate units.

Table 2 gives a compilation of the geometry of base pair stacking in A-DNA oligonucleotides averaged for the 10 different base pair stacks. The parameters listed are those of Dickerson and colleagues [26,27] and the sign convention is that adopted at the 1988 Cambridge meeting [28]. Clearly, the CpG step is unique by showing significantly reduced helical twist and propeller and a large negative slide of −2.2 Å in addition to the previously noted extended backbone conformation. There is one caveat, however: all CpG steps occur at identical positions in the sequences, namely in the center of DNA octamers crystallized nearly isomorphously in space group $P4_32_12$ [11,13–15]. Thus their conformation might be influenced by similar packing forces. In fact, very recently an A-DNA CpG step with normal stacking and backbone geometry was described [29]. Furthermore, TpA steps may also display inter-strand purine-purine stacking and a similarly extended backbone [19,20] while usually showing standard A-DNA conformation. It thus seems that pyrimidine-purine steps in general may adopt this unique conformation and that, among them, CpG steps have the highest propensity for doing so. This behaviour is reminiscent of the sequence requirement for the formation of Z-DNA where oligo d(C-G)·oligo d(G-C) sequences most readily undergo the B⟶Z transition while introducing other base pairs has a small negative effect as long as the purine-pyrimidine alternation is not disturbed [30].

3. HYPOTHESIS

Based on the above observations, we have constructed a computer model with sequence $d(C\text{-}G)_8 \cdot d(C\text{-}G)_8$ in the A form with all guanosine α and γ torsion angles *trans* (fig.2). Superficially, this model which we call A^2-DNA looks like A-DNA but there are the structural peculiarities of the 5'-CpG-3' steps with large negative slide and reduced propeller twist. Moreover, this model has a symmetry which differs from normal A-DNA because the repeat unit is a dinucleotide in-

Table 2

Properties of base-pair steps in A-DNA oligonucleotides

	AA/TT	AC/GT	AG/CT	AT/AT	CA/TG	CC/GG	**CG/CG**	GA/TC	GC/GC	TA/TA
Occurrence	–	4	2	2	1	21	**4**	3	4	4
Helical twist (°)		33.0±2.1	37.0±5.7	29.9±4.5	31.3	32.7±3.7	**22.1±4.1**	33.1±1.9	30.9±3.0	27.5±4.4
Roll (°)		3.6±3.1	5.5±0.7	1.7±0.8	10.8	5.9±3.0	**4.1±5.7**	10.0±1.0	5.6±3.9	10.3±5.5
Slide (Å)		−1.2±0.0	−1.4±0.0	−1.0±0.1		−1.7±0.4	**−2.2±0.1**	−1.7±0.4	−1.2±0.5	−1.5±0.5
Propeller (°)		−12.8±3.1	−10.5±2.6	−8.5±2.2	−11.8±2.7	−9.5±4.2	−6.3±3.0	−8.6±3.0	−10.2±4.1	−11.3±2.7

Data taken from [11,13,15–21]. The CpG step is emphasized by bold-face print

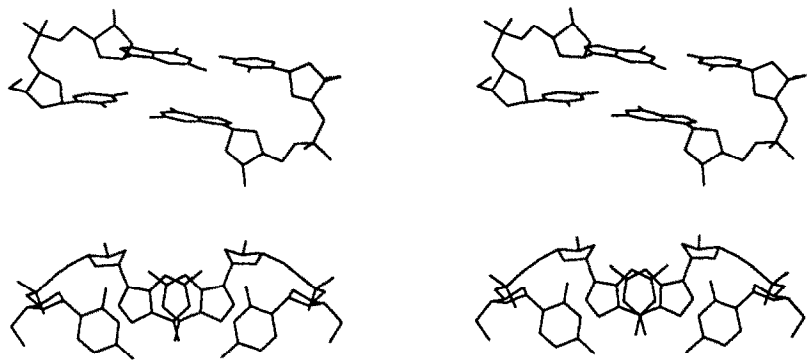

Fig.1. Two views of the central base-pair step C4-G13/G5-C12 of the crystallographically analysed A-DNA octamer d(GCCCGGGC) [11]: along the helix axis (top) and along the dyad axis (bottom).

stead of the common mononucleotide. There are 5.6 dinucleotide units per turn caused by base-pair twist angles of 23° at 5′-CpG-3′ and 41.5° at 5′-GpC-3′. At the pyrimidine-purine steps, torsion angles are α 164°, β −163.5°, γ 169°, δ 83.5°, ϵ −176°, ζ −70.5° and at the purine-pyrimidine steps torsion angles are α −80°, β −169.5°, γ 47.5°, δ 83.5°, ϵ −176°, ζ −67°.

This model is easily transformed into left-handed Z-DNA by rotating the guanosine sugars from the *anti* into the *syn* form characteristic of Z-DNA. The rotation is possible without severe sterical interference because the α,γ *trans,trans* conformation extends the

Fig.2. A model of d(C-G)$_8$·d(C-G)$_8$ in the A^2-DNA conformation. The model is based on the 5′-CpG-3′ step depicted in fig.1. Initially, a helix was constructed by stacking CpG units along the helical axis using a dinucleotide twist of 65° and a rise of 6.4 Å. This procedure gave the correct length of the P-O$_3$, bond that had to be closed in order to join the dinucleotide units. To regularize the stereochemistry of the model, several cycles with the program CORELS [31] were run where the DNA was segmented into dinucleoside pair and phosphate units with some internal degrees of freedom. This procedure relieved unreasonable clashes but preserved the symmetry and characteristics of the initial model from which the final structure deviates with a root-mean-square distance of 0.25 Å between identical atoms.

guanosine-5′-phosphate unit structurally. After rotation, α has changed to *+gauche* and γ has remained *trans* as required for Z-DNA. Moreover, the base pair is moved automatically towards the helix axis. If the conformations of the adjacent guanosines 'up' or 'down' the helix are changed the same way, the base pairs move further across the helix axis which is finally located in the minor groove (fig.3), and the sense of the double helix has changed from right-handed to left-handed. We have thus performed a smooth $A^2 \longrightarrow Z$ transition without opening or flipping of the base pairs.

We assume that these structural changes actually occur when poly d(G-C)·poly d(G-C) changes from B to Z-DNA. At low salt conditions, this polymer adopts the B-DNA form. If the salt concentration is raised, it undergoes B⟶A transformation as normally observed for mixed-sequence DNA and for DNAs with alternating or homopurine/homopyrimidine sequences except for poly d(A)·poly d(T). The structural peculiarities of the 5′-CpG-3′ steps induce the change from A to A^2-DNA which is metastable and occurs transiently. After *anti⟶syn* conversion of guanosine, it yields Z-DNA which is stable under high salt conditions. According to this proposal, the overall scheme for the B⇌Z transformation is B⇌(A⇌A^2)⇌Z with A and A^2 as transition intermediates.

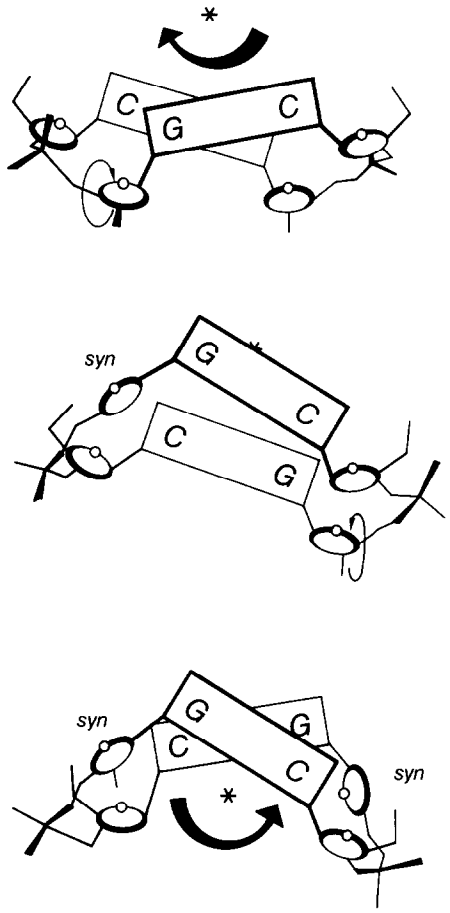

Fig.3. Schematic description of the structural transition from A^2-DNA to Z-DNA. (top) The same 5′-CpG-3′ step as illustrated in fig.1 for A^2-DNA. The upper G-C base pair is drawn heavier, the star indicates the position of the helical axis in the major groove, the right-handed screw sense is given by the thick arrow. The thin arrow at the sugar of the upper G indicates rotation into the *syn* form which pushes the G-C base pair against the helical axis (center). In the bottom picture, the sugar of the lower G is also rotated into *syn*. Both base pairs are pushed across the helical axis which is now in the minor groove and the helical sense is left-handed. Sugars are drawn as ellipsoids with ring oxygens indicated by 'O'.

There is some experimental evidence for this hypothetical scheme. In solution, only the B and Z forms are observed for poly d(G-C)·poly d(G-C). The A form was trapped in the fiber state [2,3] where it is probably stabilized by crystal packing forces. X-ray fiber diffraction clearly indicates that poly d(G-C)·poly d(G-C) adopts the A form and not A^2 which, because of the dinucleotide repeat, would require an additional reflection on the meridian. The occurrence of the A form of poly d(G-C)·poly d(G-C) in the fiber and not in solution suggests that this is a metastable state, as required in the proposed scheme. That poly d(G-C)·poly d(G-C) transforms to A-DNA if trifluoroethanol is added, suggests that in contrast to salts and normal alcohol, this reagent complexes with the A form of poly d(G-C)·poly d(G-C), thereby stabilizing this particular conformation.

One more argument for the occurrence of A^2-DNA as metastable structural intermediate is that all the oligonucleotide sequences crystallized successfully in the Z form started with d(CpGp...). The smallest unit is d(CpG) which, as ammonium salt, was found to crystallize as a mini-Z-duplex [32]. In the case of the crystalline Z-DNA hexamer d(C*p*GpC*p*GpC*p*G) [33] there are three 5′-CpG-3′ steps (marked by *p*) and the whole hexamer could occur transiently in the A^2 form whereas in the alternate sequence d(GpC*p*GpC*p*GpC) there are only two such steps. In a phosphorothioate-modified form, this oligonucleotide crystallized as a B-DNA helix [34]. The same is observed in solution where double-stranded oligonucleotides $d(G\text{-}C)_n$ (*n* going from 3 to 7) are not completely transformed into the Z form in the presence of 5 M NaCl while $d(C\text{-}G)_3$ and $d(C\text{-}G)_4$ are fully Z-DNA under these conditions [35]. The different behaviour of the two types of oligonucleotides disappears at values of *n* exceeding 8 where the ratio of the numbers of 5′-CpG-3′ and 5′-GpC-3′ steps approaches unity in either case.

Another argument for our proposal is provided by theoretical studies concerning the energies stabilizing steps CpG in DNA double helices [15,36]. In B-DNA these steps are more stable than others whereas in A-DNA they appear to be the least stable. Conflicting evidence, however, comes from a study of the melting behaviour of alternating $d(G\text{-}C)_n$ and $d(C\text{-}G)_n$ oligonucleotides [37]. These data were interpreted as indicating increased stability of 5′-GpC-3′ steps over 5′-CpG-3′ steps in B-DNA. In contrast to the scheme

for the B⇌Z transition proposed here, the same study also suggests an opening of the Watson-Crick base pairs and a separate flipping over of bases to form Z-DNA. As the interpretation is based on kinetic data obtained with a salt-jump technique which can only give indirect evidence, it should be considered with reservation, especially as it contradicts NMR experiments [6].

Last, but certainly not least, there might be spectroscopic evidence for the mechanism proposed in our model. Using Raman spectroscopy on 157 base-pair long oligo d(G-C)·oligo d(G-C), it could be shown that a band typical for $C_{3'}$-*endo* sugar pucker appears (corresponding to the A-DNA conformation or to the $C_{3'}$-*endo* form of C in Z-DNA), and that the B⟶Z transition is a two-step process [38] which we interpret with the occurrence of the 3 conformations B, A and/or A^2, and Z.

According to our model, the raison d'être for the B⇌Z transition of poly d(G-C)·poly d(G-C) resides in the stacking properties of the CpG steps when they are in the A form. The ±*gauche* ⟶ *trans* conformational change of the α and γ torsion angles of 5′-CpG-3′ opens the guanosine structurally so that the *anti* ⟶ *syn* rotation of the sugar can easily occur, leading to an obviously more stable conformation in which the open A-DNA is changed to the more compact Z-DNA. The right-to-left transition can occur smoothly, without base-pair opening, once the B⟶A conformational change is induced, with further changes A⟶A^2⟶Z-DNA. Preliminary theoretical studies have shown that the transition probably follows a zipper-like mechanism, as indicated in fig.3. Further work along these lines is in progress.

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